DATA EVALUATION RECORD

Propineb
PC Code: 522200
TXR#: 0056051
MRID 48176416

Developmental Neurotoxicity Study - Rats
OPPTS 870.6300, OECD 426

A Developmental Neurotoxicity Study with Technical Grade Propineb in Wistar Rats

Prepared for

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TXR #: 0056051

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study –Rats, Oral (Diet) (OPPTS 870.6300; OECD 426).

PC CODE: 522200 **DP BARCODE:** D396278

TEST MATERIAL: Propineb (purity 81.8-82.3% a.i.)

SYNONYMS: [[[2-[(Dithiocarboxy)amino]-1-methylethyl]carbamodithioato(2')-κS,κS']zinc].

CITATION: Gilmore, R.G. (2010). A Developmental Neurotoxicity Study with Technical Grade Propineb in Wistar Rats . Xenometrics, LLC, Stilwell, Kansas. Study Number: 08-D72-OV, June 4, 2010. MRID 48176416. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, 40789 Monheim, Germany.

EXECUTIVE SUMMARY

In a developmental neurotoxicity study (2010, MRID 48176416), Propineb (technical grade; purity 81.8-82.3%; Batch no. EDFU711100) was administered to pregnant female Wistar rats (30 rats/dose group) in the diet at nominal concentrations of 0, 30, 60 or 180 ppm from gestation day (GD) 6 through lactation day (LD) 21. The diet in ppm was adjusted during lactation to maintain a constant dose in mg/kg/day. Pups were not directly dosed. Dams were allowed to deliver naturally and were killed at weaning. The Parental (P)-generation were evaluated by cage-side and detailed clinical observations, body weight, food consumption and reproductive endpoints. On postnatal day (PND) 4, litters were culled to yield 4 pups/sex/litter (when possible) were assigned to the following sets*:* motor activity (Set A, 20/sex), auditory startle (Set B, 20/sex), passive avoidance, water maze and functional observational battery (Set C, 16/sex). On PND 21, the whole brain was collected from a separate group of (Set D; 10/sex/dietary level; for micropathologic examination and morphometric analysis).

Maternal (P-generation): Clinical signs were not considered to be treatment-related because incidence was generally low and findings were seen in the control as well as treatment groups at similar frequency. Body weight and food consumption during gestation and lactation were not different from controls at any dose level and there were no effects on litter parameters. **The maternal NOAEL is 180 ppm (equivalent to 12.3 mg/kg/day). A maternal LOAEL was not established by this study.**

Offspring (F_1 generation): In offspring, no treatment-related effects were observed on litter size, viability, clinical signs, developmental landmarks, functional observational battery, auditory startle reflex, learning and memory testing, ophthalmology, nervous system morphometric evaluation, or gross or microscopic pathology. Statistical differences in body weight, mean number of rears and brain weight (PND 75) were noted in the lowest dose group without corresponding increases in the higher doses.

In water maze performance on PND 60 (±2 days), the number of errors for the first trial of acquisition was increased (P≤0.05) for mid- and high-dose females (1.1 and 1.3 errors, respectively compared to 0.3 errors for controls). These differences were noted in the first trial of the learning phase only, occurred only in one sex, and the mean number of errors in the mid- and high-dose groups were more consistent with the historical control range for errors (0.4-1.6). Thus, it was concluded that there was no effect on learning established.

The initial mean Day 21 female pup micropathology brain measurements from the forebrain section of the brain (frontal cortex, parietal cortex, and caudate putamen) were statistically significantly *increased* at the 180 ppm dose level. However, when recuts for the low and mid dose groups were made, the low and mid dose groups were also statistically significantly increased to about the same degree (i.e. about 9-10%) without demonstrating a dose response. In conclusion, these differences were not considered to be treatment related because of a lack of dose response and possible preparation differences. **The offspring NOAEL is 180 ppm (equivalent to 12.3 mg/kg/day). An offspring LOAEL was not established by this study.**

This study is classified as **acceptable/non-guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, 83-6); OECD 426 at this time pending a comprehensive review of all available positive control data. Although the study did not demonstrate any effects of treatment, the study is still classified as acceptable since repetition of the study is not believed to contribute additional information.

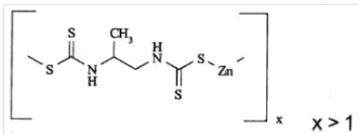
COMPLIANCE: Signed and dated Data Confidentiality (no claim of confidentiality under FIFRA, but waiver does not apply to other regulatory agencies and information is labeled trade secret), GLP Compliance, Flagging and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. Materials

1. **Test material:** Propineb (technical grade)
[[[2-[(Dithiocarboxy)amino]-1-methylethyl]carbamodithioato(2-)-κS,κS´]zinc]

Description: White-yellow powder
Lot/Batch#: EDFU711100
Purity: 81.8-82.3%
CAS #: 9016-72-2
Compound Stability: Stable at room temperature (20±5ºC)
Structure:



2. **Vehicle:** Diet.
3. **Test animals**

Species:	Rat, males and females (nulliparous and nonpregnant)	
Strain:	Wistar Crl:WI(Han)	
Age/ weight at study initiation:	At least 12 weeks of age (females) and 15 weeks of age (males) at co-housing Males: No specified weight requirement Females: 191.1 – 248.3 g	
Source:	Charles River Laboratories, Inc., Raleigh, NC	
Housing	Individually housed in suspended stainless steel cages with deodorized cage board in the bedding tray, except with one male each during co-habitation. Individually in plastic cages with corn cob bedding during gestation and lactation.	
Diet:	Purina Mills Certified Rodent Diet 5002 in meal form, *ad libitum*, except during neurobehavioral testing.	
Water:	Tap water (Kansas City Missouri Municipal Water), *ad libitum*, except during neurobehavioral testing.	
Environmental conditions:	**Temperature:**	18-26 ˚C
	Humidity:	30 – 70%
	Air changes:	Minimum daily average of 10.75 air changes / hour
	Photoperiod:	12 hr light/ 12 hr dark
Acclimation period:	At least 7 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In-life dates**
Start (initiation of treatment): January 25, 2009
End (end of in-life phase): April 24, 2009

2. **Study Schedule**
The maternal animals were mated and assigned to study when they were determined to be sperm positive. The test substance was administered via the diet from gestation day (GD) 6 through lactation day (LD) 21, at which time the pups were weaned and the dams were sacrificed. On postnatal day (PND) 4, the litters were standardized by random selection of 4 males and 4 females per litter (as closely as possible). Pups that were not selected for the F1 generation were killed and discarded. Selected offspring were sacrificed on PND 21 for brain

weight or neuropathological evaluations, and the remaining offspring were sacrificed at study termination on PND 75 (±5 days).

3. Mating Procedure

Females were paired 1:1 with males, for a maximum of five consecutive days. Each morning during the co-habitation phase, the dams and cages were examined for a vaginal plug, and vaginal smears were taken and examined for the presence of sperm. The day on which insemination was observed in the vaginal smear was designated Day 0 of gestation (GD 0) for that female. On Day 0 of presumed gestation, the female was removed and housed individually in a plastic nesting cage with corn cob bedding. Typically, females that were not sperm positive after the co-housing period or otherwise not placed on study were sacrificed without a necropsy examination.

4. Animal Assignment

Mated females were randomly assigned to the test substance, as noted in Table 1, using a stratified random sampling method based on the body weights. Female rats with body weights more or less than 20% of the mean weight were rejected. The remaining females were assigned to the control or exposure groups in sequence, as they were determined to be inseminated. P-generation males served only as breeders. As such, they had no specific weight requirements and were arbitrarily selected for co-housing with females.

Offspring were assigned to testing subgroups at the time of litter standardization on PND 4 (Table 1). An animal allocation program written in SAS was used to assign offspring to the following four sets (designated A-D) for assessment at each age. One male and/or female per litter (approximately 16 - 20 [minimum of 10]/sex/dietary level, representing at least 20 litters per level) were assigned to the following sets: motor activity (Set A), auditory startle (Set B), passive avoidance, water maze and functional observational battery (Set C). On PND 21, the whole brain was collected from a separate group of randomly selected offspring (Set D; 10/sex/dietary level; representing 20 litters per level) for micropathologic examination and morphometric analysis. The remaining pups assigned to Set D (~10/sex/dietary level) were reserved for possible use as replacement animals or were otherwise sacrificed on PND 21 without necropsy examination.

At approximately 50-60 days of age, ***randomly selected offspring animals (a minimum of 10/sex/dietary level, representing at least 20 litters per level)*** from Sets A, B and C were subjected to an ophthalmologic examination. At termination (PND 75±5 days), these animals were anesthetized and sacrificed by perfusion, with neural and muscle tissues collected for microscopic examination. Also at termination on PND 75±5 days, brains were collected from additional randomly selected animals (10/sex/dose group; representing 20 litters per level). These brains were weighed (fresh tissue weight) and discarded. The remaining animals assigned to sets A-C were sacrificed without routine gross necropsy examination or collection of tissues.

TABLE 1. Study Design					
Experimental Parameters	Set	Dose (ppm) [a]			
		0	30	60	180
Maternal Animals (P)					
No. of dams assigned	NA	30	30	30	30
Mean daily intake (mg/kg/day)	NA	0	2.3	4.4	12.3
FOB (GD 13 and 20)	C	30	30	30	30
FOB (LD 11 and 21)	C	10	10	10	10
Offspring (F_1) [b]					
Motor activity [PND 13, 17, 21, 60±2)]	A	20/sex	20/sex	20/sex	20/sex
Auditory startle habituation [PND 23, 60±2]	B	20/sex	18-20/sex	20/sex	19-20/sex
FOB [PND 4, 11, 21, 35±1, 45±1, 60±2]	C	20/sex	20/sex	20/sex	20/sex
Learning and memory [PND 23, 30, 60±2 and 7 days later]	C	16/sex	16/sex	16/sex	16/sex
Passive avoidance [PND 23 and 30]	C	16/sex	16/sex	16/sex	16/sex
Water maze [PND 60±2 and 7 days later]	C	16/sex	16/sex	16/sex	16/sex
Brain weight PND 21 PND 75±5[c]	D	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex
Neuropathology and morphometric analysis PND 21 PND 75±5	D	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex

Data extracted from page 21 of the study report.
NA - Not applicable
a Nominal concentrations were calculated using the complete isomeric total (purity 82.3%)
b Unless otherwise indicated, 1 male and/or female pup/litter was used (~16-20 [minimum of 10]/sex/dose, representing at least 20 litters).
c Remaining animals not used for neuropathology evaluations.

For FOB and motor activity testing, the same individual animals were evaluated at all scheduled time points. For the selection of animals and testing paradigms for cognitive (learning and memory) assessment, the same animals were used for assessment at the weanling and adult ages, but different tests were used at the two ages (see Observations section for details). The potential problems related to litter effects were minimized by using at least one pup/litter.

5. Dose Selection Rational

Dose levels were chosen based on the results from three studies: (1) a subchronic dietary toxicity study conducted with the test substance at dose levels of 10, 25, 100 and 400 ppm (Wirnitzer and Rosenbruch, 2003), (2) a subchronic neurotoxicity screening study utilizing doses of 30, 150 and 300 ppm (Gilmore and Lake, 2004) and (3) a 3-generation reproduction study conducted with the test substance at dose levels of 20, 60, 200 and 600 ppm (Loser, 1973).

In the subchronic dietary study (Wirnitzer and Rosenbruch, 2003), effects were only observed in the animals at **400 ppm**. Clinical signs were more pronounced in females and mainly consisted of high-stepping gait, emaciation, flaccid and soft abdominal tissues, reduced grip strength of fore- and hindlimbs. Body weights were reduced in both sexes. Gross and histopathological findings were skeletal muscle regression and skeletal muscle alterations in the thigh and in the skeletal muscle adjacent to the spinal cord, the sternum and the skin. There were no test substance-related clinical signs at lower dietary levels in either sex.

In the subchronic neurotoxicity study (Gilmore and Lake, 2004), effects were only evident in females at the **300 ppm** dietary level and included statistical decreases in body weight (11-20% decreased) and food consumption (up to 13% decreased), statistical decreases in motor and locomotor activity (up to 40% and 46% decreased, respectively) and skeletal muscle atrophy in both fore- and hind-legs. Clinical signs associated with treatment included a flat-footed, short-stepped gait affecting the hind limbs, ataxia, uncoordinated gait and righting response, decreased forelimb and hindlimb grip strength, decreased number of rears in the open field, red nasal discharge, red nasal stain. and urine stain. There were no test substance-related clinical signs at lower dietary levels in either sex.

In the reproduction study (Loser, 1973), the 600 ppm dietary level produced clear evidence of toxicity that included increased mortality in dams and statistically lower body weight and body weight gains in P-generation males and females. Clinical signs observed in P-generation animals included weak, bristled and dull coat and myasthenia of the hind extremities that considerably hindered the rats in their mobility and uptake of food. The effects were more pronounced in the females. As a consequence, the gestation rate was reduced 50% and litter size was statistically reduced 42%. Test substance-related findings in P-generation animals at *200 ppm included weak, bristled and dull coat and mild myasthenia of the hind extremities.* There were no effects related to the test substance at lower dietary levels. Body weight at birth and during the 4-week lactation period was not different from control at any dietary level for F1 generation males and females. In addition, F1 generation animals had no malformations at birth or during the lactation period at any dietary level in either sex.

Finally, a pilot study was conducted to determine whether there was evidence of exposure of the offspring by the transfer of propineb through the milk during lactation (Gilmore, Study no. 08N-P72-OJ). In this study, 12 time-pregnant Wistar rats (provided eight suitable litters - six treated and two control dams) were exposed to a nominal concentration of 0 or 180 ppm propineb in the diet from gestation Day 6 through lactation Day 14, with adjustments in dietary level during lactation to maintain a more constant dosage (mg/kg/day) throughout exposure. Offspring from each litter were sacrificed on lactation Days 4 (culls), 10 or 14 (approximately six treated/sex/age, representing six litters at each age) to measure the concentration of propineb (or active metabolites) in the milk found in the pup's stomachs. The milk was collected into an appropriate container, pooled from each litter, divided into two samples and stored in a freezer (minimum -70 ºC) until analysis. Selected milk samples from control and treated PND 14 pups were analyzed with LC/MS/MS using selected reaction monitoring (SRM). The presence of the active metabolite, phenyl urea (PU), was found in the stomach contents of PND 14 pups, which demonstrated exposure of the offspring to the test substance through the milk.

Based on these combined results, the dietary levels selected for this developmental neurotoxicity study were 0, 30, 60 and 180 ppm.

Note added by HED reviewer: The dose levels selected eventually proved to be too low since there were no obvious responses to treatment. The effects reported in the studies used to support dose selection were at 200, 300, 400 or 600 ppm. The low level of effects described at 200 ppm in the reproduction study would indicate that a dose of greater than 200 ppm should have been a dose to give a response in the high dose group.

6. Dose Administration

All doses were administered to maternal animals in the diet, on gestation Day 6 and continuing for the dams and offspring through lactation Day 21.

7. Dose Preparation and Analysis

Diet with test substance was prepared using acetone as a solvent for addition of the test substance. The acetone was allowed to evaporate prior to administration of the diet. The control diet was prepared in a similar manner but without the test substance. Formulations were prepared weekly by mixing appropriate amounts of the test substance in the diet (Purina Mills Certified Rodent Diet 5002) in meal form and were stored in the freezer (approximately -23 °C). The treated feed was provided for consumption beginning on GD 6 and continuing through lactation Day 21, with fresh feed provided every week. The control diet was prepared the same way, excluding the test substance. Dietary concentrations were not adjusted to correct for purity (percent active ingredient) in the test substance; however, concentrations were reduced by 50% during Weeks 1-3 lactation, based on estimated increases in feed consumption (g consumed/kg body wt./day) during lactation, in order to maintain a more constant level of exposure (mg/kg/day) throughout the period of exposure. A sample of each batch of feed mixed was taken and retained in the freezer until the study was complete and the analytical data deemed satisfactory. After Day 21 of postnatal development, untreated feed was provided for consumption to all F1-generation animals that were retained on study.

Concentrations of the test substance in the diet were measured using an LC-MS/MS method, using five batches of feed used in this study (See Attachment 1, page 877). The stability (at room temperature and freezer conditions) and homogeneity of the test substance in the feed were verified at dietary concentrations of 15 and 180 ppm, which bracket those used in the present study (Moore, 201).

Results

Homogeneity
The %RSD range of 3.0 to 5.3 showed the samples were homogeneous between the layers and were well within the 10 % RSD criteria for homogeneity.

Stability
The dietary concentration of 15 and 180 ppm were determined to be stable for 7 days at room temperature and 28 days at freezer conditions, and were within the criteria of ≥ 85% of the initial concentration for both levels.

Concentration (range as % of nominal): 77-84% for gestation and lactation periods.

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

C. OBSERVATIONS

1. In-Life-Observations:

a. Maternal Animals:

1) Clinical observations
P generation males and females were observed (cage-side) for clinical signs at least once daily.

2) Detailed observations
A detailed evaluation of the dams for clinical signs with a physical examination was conducted once daily from the initiation of exposure (GD 6) through LD 21. These observations were performed by an individual who was aware of the animal's dosage group assignment.

3) Functional observational battery (FOB)

Animals that were presumed to be pregnant (approximately 30 per dietary level) were subjected to a FOB on GD 13 and GD 20 and a minimum 10 dams/dietary level that were maintained on study with suitable litters were also observed on LD 11 and LD 21. All observations were performed by an individual who was unaware of each animal's dose group assignment. This evaluation was performed under standard animal room conditions (temperature, relative humidity, etc.) and included observations in the home cage, during handling and outside the home cage in an open field (one minute), using standardized procedures. Since it was not feasible for one person to evaluate all animals on all test occasions, the laboratory maintains evidence of inter-observer reliability for individuals who were involved with performing these observations (Sheets, 2004; 1993). This observational battery included, but was not limited to, assessments (with severity scoring) of lacrimation, salivation, piloerection, exophthalmia, urination, defecation, pupillary function, palpebral closure, convulsions, tremor, abnormal movements, unusual behaviors and posture and gait abnormalities.

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalmia, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth and any other observations that may facilitate interpretation of the data.

4) Body Weight and Food Consumption

Body weight and food consumption were measured once weekly during gestation and lactation, as follows: GD 6-13 and 13-20; LD 0-7, 7-14 and 14-21. In addition, dams were weighed on GD 0 and LD 4. Body weight gain was reported for GD 0-20. Measures of food consumption may have included consumption by the pups, especially during the third week of lactation.

5) Delivery and Culling

Each dam was evaluated daily for evidence of delivery from GD 20 to the completion of delivery, which was designated LD 0 for the dam and PND 0 for the pups. Litter size (the number of pups delivered) and pup status (live or dead) at birth were recorded for each litter. If a dam delivered fewer than three pups per sex or if the litter size decreased to fewer than seven pups by PND 4, the dam and litter were sacrificed without necropsy examination. For litters that met the minimum size requirements, the size of each litter was adjusted on PND 4 to yield, as closely as possible, four males and four females. If there were more than 23 acceptable litters for any dietary level, the surplus litters were sacrificed on PND 4 after weighing without routine necropsy, with preference given to retaining litters with a full complement of four males and four females. Culled dams and pups were sacrificed by carbon dioxide (CO_2) asphyxiation and decapitation, respectively. Dams with insufficient litters were also sacrificed by CO_2 asphyxiation.

6) Termination

P generation males and females were sacrificed by CO_2 asphyxiation. A gross necropsy examination was not performed on P generation animals. Following co-habitation, males were sacrificed and discarded. Dams were sacrificed on LD 21, following the weaning of their respective litters. Females that were sperm positive and/or had an internal vaginal plug, but did not deliver, were generally sacrificed on GD 24 without necropsy examination.

b. Offspring

1) Litter Observations

The day of completion of parturition was designated as Lactation Day (postnatal Day) 0. Live pups were counted, sexed and weighed individually for each litter on postnatal Days 0, 4, 11, 17, and 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. More detailed observations for clinical signs were made once daily before weaning and once weekly thereafter.

2) Developmental Landmarks

Beginning on PND 38, male offspring were examined daily for balanopreputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. Beginning on PND 4, selected males and females were tested daily for surface righting. The age of onset was recorded for each of these landmarks. On PND 21, all pups were tested for the presence of pupil constriction.

4) Body Weight and Food Consumption

Live pups were weighed individually for each litter on PND 0, 4, 11, 17 and 21, and once weekly thereafter. The individual pups were also weighed when vaginal potency or balanopreputial separation were first evident. Food consumption was not measured after weaning on PND 21.

5) Neurobehavioral Evaluations

Observations and the schedule for those observations are summarized as follows. The test rooms used for motor activity, auditory startle habituation and passive avoidance conditioning were standard animal rooms that were set to be maintained on the same light-dark cycle as the room in which animals were housed, with tests conducted during the light phase. The water maze testing was performed in the room where animals were housed. The order of testing and assignment of animals to specific test devices was semi-random, such that groups were balanced across test times and devices and no animal was tested more than once in the same device. One planned exception was that animals were purposely tested in the same water maze on both occasions, as per standard procedure. Males and females were generally tested on the same days at the appropriate days of age. After sexual maturation, test devices were cleaned during the ensuing interval to reduce the residual scent from the other gender.

i) Functional Observational Battery (FOB)

On PND 4, 11, 21, 35 (±1 day), 45 (±1 day) and 60 (±2 days), approximately 20 offspring/sex/group (representing at least 20 litters per level; Subset C) were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage being observed. This evaluation was performed according to the procedures described for maternal animals (see above). The only difference was that the neonates (i.e., PND 4 and 11) were not evaluated in the open field.

ii) Motor Activity Testing

Motor activity was measured for approximately 20 rats/sex/dose (representing at least 20 litters per level; Subset A) on PND 13, 17, 21 and 60 (±2 days). The same offspring were evaluated in the figure-eight maze for 60 min (six 10 min intervals) at each time point, using a computer-automated system (Universal Maze Monitoring System, Version 1.41, Columbus Instruments, Columbus, OH) and personal computer for automated data collection. The figure-eight maze was selected as an established and widely used automated

activity device that can be used to detect both increases and decreases in activity (Reiter, 1983). Each maze consisted of a series of inter-connected alleys (approximately 10 x 10 cm in cross-section) converging on a central arena and covered by transparent acrylic plastic. Each maze had eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys) to measure activity and an activity count was registered each time a beam was interrupted. The floor of each maze rested above absorbent paper, which was changed routinely at the end of each day. A Columbus Instruments (Columbus, OH) Universal Maze Monitoring System and a personal computer were used for automated data collection. Broad-spectrum background noise [74± 2 dBA)] was provided throughout the test to minimize acoustical variations during testing. The uniformity of light intensity (100±70 Lux) over each maze was verified daily. Motor and locomotor activities were examined as total activity counts (beam interruptions) for the 60-min session and as activity during each ten-min interval. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii) <u>Auditory Startle Reflex Habituation</u>

Auditory startle reflex habituation testing was performed in approximately 20 rats/sex/dose (representing at least 20 litters per level; Subset B) on PND 23 and 60 (± 2 days), using an automated system. A personal computer was used to control the operation of an integrated startle response test system (Coulbourn Acoustic Startle, Version 3.210-00, Coulbourn Instruments, Allentown, PA) and for automated data collection. Groups of four animals (maximum) were tested simultaneously within each of two startle system enclosures. Each enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material, and houses a speaker mounted in a central position within the ceiling of the enclosure to provide the eliciting stimulus (S2) - a 50-msec burst (0 msec rise/fall) of broad-spectrum "white" noise [118 ± 3dB(lin)]. Each enclosure also houses four load cell/force transducer assemblies that are designed to measure the startle response. During the test session, animals were placed into individual restraining cages that were positioned on top of each load cell. The test session consisted of 50 trials that began following approximately a 5-min adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10-sec intervals. The peak response amplitude was determined for each trial as described below. The average response amplitude and the magnitude of decrease (habituation) over blocks of ten trials were compared among the dosage groups. Data collection began with the presentation of S2 and continued thereafter for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (i.e., one sample/msec for 200 msec) and converted to grams using a previously determined calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's individual response curve. Baseline was defined as the average force (g) exerted on the platform during the first 8 msec following the onset of S2, a time period that precedes response onset. This baseline value was taken to represent an approximate body weight measurement that was used to verify that the equipment used to measure the response amplitude was functioning properly. Response amplitude is defined as the maximum value of the average curve, minus the baseline (i.e., removing the animal's body weight from the measurement). Latency to peak is the time (msec) following the onset of S2 when the peak response amplitude occurs.

iv) <u>Learning and Memory Testing</u>

Learning and memory testing was performed in approximately 16 rats/sex/dose (minimum 10 offspring/sex/dose; Subset C). ***The same set of animals was used for testing passive***

avoidance (on PND 23 and 30) and water maze (PND 60 ± 2 days and again seven days later).

Postweaning - Passive Avoidance

Animals were tested for acquisition on PND 23 and for retention on PND 30. Testing was conducted using equipment and computer programs from Coulbourn Instruments (Graphic State Notation 2 Version 2.002-00, Allentown, PA). Testing took place in individual isolation cubicles, each housing a single shuttle cage. Each isolation cubicle was lined with foam insulation to attenuate sound in the chamber and had a fan with a baffled air intake and exhaust system for ventilation. The shuttle cage consisted of a Plexiglas and stainless-steel rectangular chamber fitted with front-loading access. Each shuttle cage (15 inches wide x 7.25 inches deep) was separated into two compartments of equal size (approximately 7 x 7 inches) by a wall that supported a centrally-located sliding (guillotine-type) door. The two compartments were identical, except that the walls in one compartment were lined with black film (dark-side) and the walls in the other compartment were not lined and it was illuminated during the test with a high-intensity lamp. The lamp was switched on to illuminate the light compartment at the start of each trial and remained on until either the animal crossed to the dark compartment or the trial ended. The floor of the cage consisted of a grid of stainless-steel bars. The movement of the animal from the starting (light) side to the dark compartment was detected by a photocell system. A Coulbourn solid-state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) distributed shock to the grid floor when the animal crossed to the dark compartment.

After adaptation, individual animals were placed individually into the "lighted" compartment of a conditioning apparatus (the shuttle cage), facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the non-illuminated side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered and the light switched off - signaling the end of that trial. At that time, the animal was returned promptly to the holding cage to wait for the next trial. If the rat failed to cross within 180 sec, it was returned to the holding cage and the latency assigned an arbitrary score of 180. This restriction dictated the use of nonparametric statistical analyses. The procedure was repeated until either the rat remained in the lighted compartment for 180 sec on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For this second trial, rats were placed in the illuminated side of the apparatus, given a 20-sec acclimation period and the latency to enter the dark side recorded. Animals that either failed to reach criterion performance within 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase of the experiment. The dependent measures were the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2 (learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Adult (PND 60) Offspring - Water Maze

Animals were tested on PND 60 (±2 days), and again seven days later. Only animals that demonstrated acquisition were tested for retention. The water in the M-maze was maintained at 22 ± 1°C. The mazes were constructed of opaque Plexiglas, with corridors approximately five inches wide and walls approximately 16 inches high with approximately 7.5 inches of water. This maze was selected as an established and widely-used device that can be used to measure associative learning and memory.

On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first (learning) trial, the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (±5) seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Animals that satisfied the above criteria within the 15-trial limit were tested for retention seven days following acquisition (animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment). The correct goal and the criterion were the same for both sessions. Dosage groups were compared for the following dependent measures: Measures for acquisition included the number of trials-to-criterion, the average number of errors (incorrect turns in the maze) for each trial and the latency (in seconds) to reach the correct goal on trial 2 (a measure of short-term retention). Measures for retention included the number of trials-to-criterion, the average number of errors for each trial and the latency (in seconds) to reach the correct goal on trial 1 (a measure of long-term retention).

6) Ophthalmology

At approximately 50-60 days of age, ophthalmic exams were conducted using the males and females (a minimum of 10/sex/dietary level; representing at least 20 litters per level) that were selected for perfusion at study termination. If needed to clarify the significance of findings, the animals reserved for adult brain weight measurements were also subjected to ophthalmologic examination. The exam took place in a semi-darkened room. The pupillary reflex was tested using a penlight or transilluminator, with a mydriatic agent applied to each eye to dilate the pupil. The conjunctiva, cornea and lens were examined with a slit lamp microscope either before or after pupillary dilatation. After mydriasis, the vitreous humor, retina, choroid and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

7) Postmortem Observations

a. Maternal Animals

Maternal animals were sacrificed by CO_2 asphyxiation on LD 21 following the weaning of their respective litters. The dams were discarded without postmortem examination. Females that were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed on GD 24 without necropsy examination.

b. Offspring

Necropsy

The offspring selected for brain weight or neuropathological evaluations were sacrificed on PND 21 (Subset D) or 75±5 days (Subset A, B and C). F1 generation animals that were found moribund (if any) while on study were sacrificed by CO_2 asphyxiation and underwent a gross necropsy examination. In addition, randomly selected animals from Sets A-C that were used to measure fresh brain weight on PND 75±5 days were sacrificed by CO_2 asphyxiation and underwent a necropsy examination. Where required, the necropsy involved an examination of all organs (including the brain), body cavities, cut surfaces, external orifices and surfaces, with all gross abnormalities recorded. Gross lesions in neural tissues or skeletal muscle were appropriately sampled for microscopic examination. Other gross lesions were generally not collected for microscopic examination. Animals found dead (if any) underwent a necropsy examination and were disposed of without the routine collection of tissues.

Perfusion

Animals that were selected for perfusion on PND 21 (from Set D) or at study termination (from Sets A-C) were deeply anesthetized using an intraperitoneal dose of pentobarbital (approximately 50 mg/kg) and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by *in situ* fixation using universal fixative (1.0% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde) in phosphate buffer. On PND 21, only the brain (with olfactory bulbs) was collected. At study termination, the brain and spinal cord, eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle and both forelimbs were collected. All tissues were post-fixed in 10% buffered formalin. The brain was weighed upon removal from the skull, prior to placement into formalin, and the brain to body weight ratio calculated.

Measurements

Prior to sectioning the brain for histology, a Vernier caliper was used to obtain two linear measurements (mm).

1. Anterior-to-posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and
2. Anterior-to-posterior (AP) length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole.

These gross measurements were performed on 10 rats/sex/dose at PND 21 and 75±5 by an individual who was aware of dose group assignments.

Histology

Neuropathological examination was scheduled for rats in Set D (10 rats/sex/dose) at PND 21 and in sets A-C at PND 75±5. The brain tissue from perfused animals and any gross lesions collected at necropsy were further processed for microscopic examination. After the gross measurements were taken, the brain was divided into eight coronal sections for microscopic examination. The eight brain sections were processed according to standard procedures for paraffin embedding, sectioned at approximately 5 m and examined after staining with hematoxylin and eosin (H&E). In addition, the brain sections reserved for morphometric measurements (levels 3-5 and 7) were stained using luxol fast blue/cresyl violet. Additional tissues were collected for microscopic examination from animals that were perfused at study termination. These included three levels of the spinal cord (cervical, thoracic and lumbar), the cauda equina, eyes, optic nerves and gastrocnemius muscle which

were embedded in paraffin and stained with H&E. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol methacrylate (GMA). GMA-embedded tissues were sectioned at 2 µm - 3 µm and stained using a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section. The CHECKED (X) tissues were evaluated for adult offspring.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES**
X	Forebrain	X	Sciatic
X	Center of cerebrum	X	Tibial
X	Midbrain	X	Sural
X	Cerebellum		**OTHER**
X	Pons	X	Lumbar dorsal root ganglion
X	Medulla oblongata	X	Lumbar dorsal root fibers
	SPINAL CORD	X	Lumbar ventral root fibers
X	Cervical swelling	X	Cervical dorsal root ganglion
X	Thoracic	X	Cervical dorsal root fibers
X	Lumbar swelling	X	Cervical ventral root fibers
	OTHER	X	Gastrocnemius muscle
X	Gasserian ganglion		
X	Cauda equine		
X	Optic nerve		
X	Eyes		

Micropathology and Morphometry

The tissues from high-dose animals were examined relative to those from the respective control group. If no treatment-related lesion was evident, further analysis was not performed. Any region where treatment-related neuropathology was observed underwent the following semi-quantitative analysis. Sections from all dose groups were coded and examined in randomized order without knowledge of the code. The frequency of each type of lesion was determined with the severity of each lesion graded.

Selected brain regions underwent the following quantitative analysis, with the individual performing the measurements aware of dose assignments. Initially, eight linear measurements were taken. If treatment-related effects were evident following this initial evaluation, then additional measurements may have been undertaken. Two of the seven measurements involved gross measurements of the intact brain, as described above. The other five were taken from the histologic sections using software calibrated with an ocular micrometer. These five measurements are described as follows:

1. Frontal cortex thickness (forebrain). This measurement was of the dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm.

2. Parietal cortex thickness (forebrain). This measurement was of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.

3. Caudate putamen horizontal width (forebrain; maximum cross-sectional width). This measurement was performed on the coronal section taken at the level of the optic chiasm.

4. Hippocampal gyrus thickness (midbrain). This measurement was of the full width of the

hippocampal gyrus from the ventral tail of the dentate gyrus to the overlying subcortical white matter. Measurements were taken from the hippocampus from both sides of this section and the mean value was recorded.

5. Cerebellum height (cerebellum / pons). This measurement extended from the roof of the fourth ventricle to the dorsal surface.

In addition to these measurements, all brain sections from these control and high-dose male and female offspring underwent an extensive micropathologic evaluation. Morphometric data were collected on 10 rats/sex/dose at PND 21 and 75±5.

D. DATA ANALYSIS

1. Statistical Analyses

Group means were compared at the 5% and 1% levels of significance, with the exception of Bartlett's test, which was compared at the level of 0.1%. Statistical analyses were performed using software from INSTEM Computer Systems, SAS and TASC. In general, continuous data were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using an analysis of variance (ANOVA), followed by Dunnett's test when appropriate. In the event of unequal variances, Kruskal-Wallis test was used, followed by the Mann-Whitney U test when appropriate. Furthermore, additional statistical tests to assess continuous and frequency pathological data may have been used when deemed appropriate.

PARAMETER	STATISTICAL ANALYSES
Continuous FOB data	Data were analyzed by analysis of variance (ANOVA), with post-hoc comparisons using Dunnett's test.
Categorical FOB data	Data were analyzed using General Linear Modeling and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively.
Motor and locomotor activity	Session activity data for the four test occasions were first analyzed using an ANOVA to determine whether there was a significant day by treatment interaction. For days on which there was a significant treatment effect, Dunnett's test was used to determine whether the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine whether there was a significant treatment by interval interaction on each test occasion. For those test days, the data for each interval was subjected to analysis using Dunnett's test to determine whether the treated group was significantly different from the control.
Auditory startle response amplitude	Data (peak amplitude) for the two test occasions were first analyzed using an ANOVA procedure. If there was a significant group effect, Dunnett's test was used to determine whether the treated group was significantly different from control. The response amplitude data for each block of ten trials (five blocks/test session) were subjected to a Repeated-Measures ANOVA, using test block as the repeated measure. If there was a significant group by block interaction, the values for each block were subjected to analysis using Dunnett's test to determine if the results for treated animals were significantly different from control.
Passive avoidance	Latency data were analyzed using a Wilcoxon Test for time to failure (i.e., time to cross). The number of trials-to-criterion was analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning (acquisition) phase was analyzed as incidence data.
Water maze	Latency data were analyzed by a univariate ANOVA, with post-hoc analysis using Dunnett's test. The number of trials-to-criterion and the number of errors were analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning phase was analyzed as incidence data.

PARAMETER	STATISTICAL ANALYSES
Organ weights Gross brain weights	Bartlett's test was performed on the data, followed by ANOVA (parametric) or Kruskal-Wallis (nonparametric), as appropriate.
Microscopic brain weights	ANOVA and/or t-tests were performed.
Ophthalmology Gross pathology	Data were first visually screened and if potential compound effects were suspected, then Chi-Square and one-tailed Fisher's Exact tests were used.
Micropathology	Chi-square Fisher's exact test was performed.

These statistical analyses are considered appropriate.

2. Indices

a. Reproductive indices

The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating Index = No. of inseminated females / No. of females co-housed with males x 100

Fertility Index = No. of pregnant females / No. of inseminated females x 100

b. Offspring viability indices

The following viability (survival) indices were calculated from lactation records of litters in the study:

Live Birth Index = No. of live pups born per litter / Total no. of pups per litter x 100

Viability Index = No. of live pups on day 4 pre-culling per litter / No. of live pups born per litter x 100

Lactation Index = No. of live pups on Day 21 per litter / No. of live pups on day 4 post-culling per litter x 100

3. Positive and Historical Control Data

References were provided to previous studies to document the positive control data base for the testing laboratory as follows:

- Pathology positive control data (***Sheet and Lake, 2001***)
- Inter-observer reliability (agreement) for individuals who were involved with performing these observations ***(Sheets, 2004; 1993***)
- Increase (triadimefon) and decrease (chlorpromazine) activity were conducted to verify the sensitivity, reliability and validity of these test procedures ***(Sheets, 1993; 2002).***
- Test norms for the appropriate ages under these conditions and the effects of perinatal exposure to a reference chemical (methimazole) on activity in animals tested at these ages **(Sheets and Lake, 2001).**
- `The adequacy of the auditory startle test procedures has been established by performing studies with untreated animals and with rats treated with reference substances ***(8OHDPAT and mCPP***) that alter startle response amplitude (Sheets, 2001). The adequacy of the passive avoidance test procedures has been established by performing studies with untreated animals and with rats treated with a reference substance (scopolamine) that interferes with acquisition and/or retention (***Sheets, 2001; Bammer, 1982***). The adequacy of the water maze test procedures was also established by performing studies with untreated animals and rats treated with a test substance (scopolamine) that interferes with acquisition and/or retention (Sheets, 2001).

A full review of these studies to satisfy the establishment of a contemporary positive control data base is pending policy on review of such studies.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and Clinical and Functional Observations:

Mortality and moribundity
No maternal mortality or moribundity occurred at any dose level during the gestation and lactation periods.

Clinical observations:

Gestation
There were no test substance-related clinical signs at any dose levels. The following clinical signs were observed: red vaginal discharge on GD 13 and bend in tail (1 high-dose dam each), missing digits (1 mid-dose dam) and hair loss (1 to 3 dams from each dose group, including controls) (Table 2). These findings were considered incidental and not treatment-related because they are common findings associated with nest-building behavior in pregnant rats and are observed with similar incidence levels in controls.

Lactation
There were no treatment-related effects in clinical signs at any dose levels during the lactation period. Clinical signs observed included red vaginal discharge
(1 high-dose dam on LD 1), lacrimal stain (1 high-dose dam on LD 15 and 16), nasal stain and dehydration (1 high-dose dam on LD 18) and areas of hair loss (2-3 dams at all dietary levels, including control) (Table 2). None of these findings are considered to be related to the test substance since incidence was generally low (only seen in 1 animal on 1 or 2 days) and findings are seen in controls as well as treated animals.

TABLE 2. Summary Data for Maternal Clinical Observations During Gestation and Lactation

Observation	Dose (ppm)			
	0	**30**	**60**	**180**
Gestation (Days 6-24)				
No. of females examined on GD 6	30	30	30	30
Red Vaginal Discharge	0	0	0	1
Hair Loss	2	3	1	2
Missing Digits	0	0	1	0
Ben in Tail	0	0	0	1
No. of Females Found Dead	0	0	0	0
Lactation (Days 0-21)				
	0	**30**	**60**	**180**
No. of females examined on LD 0 [a]	29	30	29	29
Lacrimal Stain	0	0	0	1
Nasal Stain	0	0	0	1
Hair Loss	2	3	3	2
Red Vaginal Discharge	0	0	0	1
Dehydration	0	0	0	1
No. of Females Found Dead	0	0	0	0

[a] The numbers of dams with no pups by GD 24 were 1, 0, 1, and 1 for the controls, low-, mid-, and high-dose groups, respectively.

Values are the number of rats with the findings.
Data were obtained from pages Tables 2 (page 70) and 5 (page 76-77) in the study report.

Functional observational battery (FOB): There were no treatment-related findings in dams at any dose level during gestation and lactation periods. As shown in Table 3, handling observations showed red vaginal discharge in one mid and high-dose dam, each on GD13, and one low-dose dam on LD 21. Additionally, areas of hair loss described as alopecia were reported for 1-2 dams at all dose levels, including controls during gestation (GD 13 and 20) and lactation (LD 11 and 21) (Table 3). These findings were seen in controls as well as treated animals; therefore, they are considered incidental and could not be attributed to the administration of test substance.

TABLE 3. Summary Data for Functional Observational Battery in Dams

Observation	Dose (ppm)			
	0	30	60	180
Gestation Day 13				
Number of Animals Examined:	30	30	30	30
Handling-Other, Alopecia				
Not Observed	29(97)	29(97)	30(100)	28(93)
Present	1(3)	1(3)	0(0)	2(7)
Handling-Red Vaginal Discharge				
Not Observed	30(100)	30(100)	29(97)	29(97)
Present	0(0)	0(0)	1(3)	1(3)
Gestation Day 20				
Number of Animals Examined:	30	30	30	30
Handling-Other, Alopecia				
Not Observed	28(93)	28(93)	29(97)	29(97)
Present	2(7)	2(7)	1(3)	1(3)
Lactation Day 11				
Number of Animals Examined:	10	10	10	10
Handling-Other, Alopecia				
Not Observed	9(90)	10(100)	10(100)	9(90)
Present	1(10)	0(0)	0(0)	1(10)
Lactation Day 21				
Number of Animals Examined:	10	10	10	10
Handling-Other, Alopecia				
Not Observed	8(80)	10(100)	9(90)	8(80)
Present	2(20)	0(0)	1(10)	2(20)
Handling-Red Vaginal Discharge				
Not Observed	10(100)	9(90)	10(100)	10(100)
Present	0(0)	1(10)	0(0)	0(0)

Findings were not statistically different from control, $p \leq 0.05$
Values represent the number of animals and % incidence (in parentheses) with or without observation
Data were obtained from Table 16 on pages 115-138 of the study report.

2. Body Weight and Food Consumption

Group mean body weights and food consumption values for pregnant and nursing dams are presented in Table 4.

Body weight

No statistically significant differences in body weight and body weight gains were observed at any dose level during gestation or lactation periods.

Food consumptions

Food consumption was not statistically significantly different from controls for any dose during either the gestation or lactation period.

TABLE 4. Mean (±SE) Maternal Body Weight and Food Consumption					
Observation/study week		Dose (ppm)			
		0	30	60	180
Gestation					
Mean body weight (g)	GD	221.7±2.65 (29)	218.9±2.83 (30)	221.4±2.38 (29)	217.9±3.02 (29)
Mean body weight (g)	GD	243.1±2.99 (29)	244.4±2.69 (30)	246.6±2.61 (29)	243.6±2.82 (29)
Mean body weight (g)	GD 1	269.0±3.96 (29)	271.4±3.04 (30)	272.3±2.74 (29)	270.0±3.38 (29)
Mean body weight (g)	GD 2	335.7±4.39 (29)	333.1±4.01 (30)	336.5±4.32 (29)	322.9±4.81 (29)
Mean body weight gain (g)	GD 0-2	114.0±2.89 (29)	114.2±2.35 (30)	115.1±3.13 (29)	105.0±3.42 (29)
Mean food consumption (g/animal/day)	GD 6-1	20.6±1.83 (29)	20.9±1.03 (30)	19.8±0.69 (29)	18.2±0.55 (29)
Mean food consumption (g/animal/day)	GD 13-2	21.1±0.62 (29)	22.0±1.28 (30)	21.7±0.63 (29)	20.4±0.63 (29)
Lactation					
		0	**30**	**60**	**180**
Mean body weight (g)	LD	261.6±3.32 (29)	262.7±3.55 (30)	259.8±3.42 (29)	252.5±3.35 (29)
Mean body weight (g)	LD	279.7±3.63 (27)	280.7±3.67 (29)	277.8±3.51 (29)	268.1±4.16 (27)
Mean body weight (g)	LD	286.9±3.73 (23)	289.4±3.95 (23)	284.3±4.10 (23)	277.4±3.74 (23)
Mean body weight (g)	LD1	303.1±4.41 (23)	309.3±4.16 (23)	306.9±4.19 (23)	299.6±3.76 (23)
Mean body weight (g)	LD 2	296.5±4.69 (23)	297.1±3.19 (23)	291.2±3.01 (23)	284.2±3.14 (23)
Mean food consumption (g/animal/day)	LD 0-	42.4±2.21 (23)	40.6±2.42 (22)	40.1±3.11 (23)	37.3±1.94 (23)
Mean food consumption (g/animal/day)	LD 7-1	56.9±1.28 (23)	61.2±3.35 (23)	55.7±1.32 (23)	54.9±1.52 (23)
Mean food consumption (g/animal/day)	LD 14-2	73.0±2.43 (23)	74.3±2.07 (22)	68.5±1.70 (23)	70.3±2.84 (23)

Values are mean ± standard error
(#) = Number of animals
Means for gestation period include only dams known to deliver pups (either alive or dead).
Values were not statistically different from control, $p \leq 0.05$ or $p \leq 0.01$
Data were obtained from Tables 3 (page 71), 4 (page 73), 6 (page 78) and 7 (page 80) of the study report

3. Test Substance Intake

Based on maternal food consumption, body weights and nominal dietary concentrations, the doses expressed as mean daily mg test substance per kg body weight during the gestation and lactation periods are presented in Table 5. The average daily intake of active ingredient was 0, 2.3, 4.4 and 12.3 mg/kg/day for the nominal concentrations: 0, 30, 60 and 180 ppm, respectively.

TABLE 5. Mean (±SE) Maternal Test Substance Intake (mg/kg body weight/day)			
Period	Dose (ppm)		
	30	60	180
Gestation			
Gestation Days 6-13	2.1±0.10 (30)	3.7±0.13 (29)	10.5±0.27 (29)
Gestation Days 13-20	2.0±0.12 (30)	3.7±0.09 (29)	10.5±0.22 (29)

TABLE 5. Mean (±SE) Maternal Test Substance Intake (mg/kg body weight/day)			
Period	**Dose (ppm)**		
	30	**60**	**180**
Lactation			
	30	**60**	**180**
Lactation Days 0-7	1.9±0.12 (22)	4.0±0.36 (23)	10.3±0.54 (23)
Lactation Days 7-14	2.7±0.14 (23)	5.0±0.13 (23)	13.9±0.34 (23)
Lactation Days 14-21	3.0±0.09 (22)	5.7±0.16 (23)	16.5±0.67 (23)

Values are mean ± standard error
(#) = Number of animals
Dietary concentrations were reduced during weeks 1-3 of lactation by 50% based on estimated increases in feed consumption (g consumed/kg body wt./day) during lactation.
Nominal concentrations were calculated using the complete isomeric total (purity 82.3%)
Data were obtained from Table 8 on pages 83-84 of the study report

4. Reproductive Performance

Results for the maternal animals are summarized from the report in Table 6. There were no treatment-related effects on reproductive parameters at any dose level.

TABLE 6. Summary Data for Reproductive performance				
Observation	**Dose (ppm)**			
	0	**30**	**60**	**180**
No. of Animals Co-housed [a]	30	30	30	30
No. of Animals Mated	30	30	30	30
Maternal Wastage				
No. of Dams Not Pregnant	1	0	1	1
No. of Dams that Delivered Dead Pups	0	0	0	0
No. of Dams with Pre-Mature Delivery	0	0	0	0
Mating Index	100.0	100.0	100.0	100.0
Fertility Index (No. of pregnant females/No. of inseminated females X 100)	96.7	100.0	96.7	96.7
Gestation Length (days) [b]	21.9±0.07 [22.0] (21.0-22.0)	22.0±0.09 [22.0] (21.0-23.0)	21.7±0.09 [22.0] (21.0-22.0)	21.7±0.09 [22.0] (21.0-22.0)

[a] Number of animals assigned to each dietary level.
[b] Values are mean ± standard error, [median], and (range).
Values were not statistically different from control, $p \leq 0.05$ or $p \leq 0.01$
Data were obtained from Tables 1 (page 67) and 6 page 41) of the study report.

5. Maternal Postmortem Results

Not applicable to the present study.

B. OFFSPRING (F_1 GENERATION):

1. Viability and Clinical Signs

Litter size and viability (survival) results from pups during lactation are summarized in Table 7. There were no treatment-related effects on offspring litter size or viability at any dose level.

On PND 0-21, there were no test substance-related clinical signs during lactation in males or females at any dose level. One mid- and one high-dose pup each had extra digits. This finding was not considered to be related to treatment since this finding was not seen in the multigeneration study conducted at much higher dietary levels (Loser, 1973) or in the two-

generation reproduction study conducted in Xenometrics laboratory at the same dietary levels (Young, Study no.08-R72OB). Additional incidental findings that were evident on occasion in a few (1-3) individuals from various dose groups, including controls, were bruising (including at time of delivery), wound or cuts, scab and ocular opacity. These findings are common in such young rats and did not occur in a dose-related pattern to indicate a relationship with exposure to the test substance.

There were no test substance-related clinical signs after weaning at any dose level. Clinical findings observed in control and/or treated animals from various dose groups included yellow perigenital stain, brown perianal stain, red lacrimal and nasal stains, clear lacrimation, areas of alopecia and/or thinning hair, exophthalmia, ocular opacity, maloccluded teeth and minor dermal lesions described as scabs. These findings were considered incidental and not treatment-related since they did not occur in a dose-related pattern and were seen in controls. There were no missing, found dead or moribund offspring (males and females combined) observed after culling litters on PND 4.

TABLE 7. Summary of Litter Size and Viability Data During Lactation

Observation	Dose (ppm)			
	0	30	60	180
No. of Litters	23	23	23	23
Total No. of Pups Born	274	262	259	257
Total No. of Pups Missing	0	1	2	0
Litters with Pups Missing	0	1	2	0
Total No. of Pups Found Dead	0	0	0	0
Litters with Pups Found Dead	0	0	0	0
Total No. of Pups Cannibalized	0	0	0	0
Litters with Pups Cannibalized	0	0	0	0
Litter Size	11.9±0.32 [12.0] (9.0-15.0)	11.4±0.35 [11.0] (9.0-16.0)	11.3±0.43 [11.0] (7.0-15.0)	11.2±0.49 [11.0] (7.0-15.0)
Stillborn pups: Number	0	0	0	0
Mean No. of Viable Pups Day 0 (birth) Day 4 (Pre-cull) [a] Day 4 (Post-cull) [b] Day 21	 12 12 8 8	 11 11 8 8	 11 11 8 8	 11 11 8 8
Live birth index [c]	100±0.0 [100] (100-100)	100±0.0 [100] (100-100)	100±0.0 [100] (100-100)	100±0.0 [100] (100-100)
Viability index [c]	100±0.0 [100] (100-100)	99.7±0.33 [100] (92-100)	99.3±0.51 [100] (91-100)	100±0.0 [100] (100-100)
Lactation index [c]	100±0.0 [100] (100-100)	100±0.0 [100] (100-100)	100±0.0 [100] (100-100)	100±0.0 [100] (100-100)

[a] Before standardization (culling).
[b] After standardization (culling).
[c] Values are mean ± standard error.
Values were not statistically different from control, $p \leq 0.05$ or $p \leq 0.01$
Data were obtained from Table 9 (pages 86-87) of the study report

2. Body Weight:

Preweaning body weight

Mean preweaning pup body weight data are presented in Table 8. Offspring body weights during lactation were not affected by the test substance at any dose level in either sex. However, significant increases ($p \leq 0.05$) in offspring body weights were noted in both sexes at the dose level of 30 ppm on PND 0 (↑7% in both sexes) and PND 4 (↑7-8% in males pre- and post culling and ↑7% in females pre-culling). In addition, body weight was significantly ($p \leq 0.05$) increased in the male 30 ppm group on PND 21 (↑5%). For all remaining time points, body weights in the 30 ppm males and females were increased (4% in males and 4-7% in females), but they were not statistically significant.

Body weight *gain* was not considered to be affected by the test substance at any dietary level in either sex. Body weight gain for PND 11-21 was statistically increased in low-dose males (↑7%), and in males and females combined (↑6%). There was a similar non-statistical trend in body weight gain for PND11-21 in low-dose females (5%) as well. These differences from control in body weight and body weight gain are not considered to be test substance-related since there was no relationship to dose and were only seen in low-dose animals.

TABLE 8. Mean (±SE) Pre-weaning Pup Body Weights (g)

Postnatal Day	Dose (ppm)							
	0	30	60	180	0	30	60	180
	Males				Females			
0	6.0±0.06 (23)	**6.4*±0.10 (↑7%) (23)**	6.1±0.10 (23)	5.9±0.06 (23)	5.6±0.06 (23)	**6.0*±0.10 (↑7%) (23)**	5.7±0.09 (23)	5.6±0.08 (23)
4 [a]	10.2±0.14 (23)	**11.0*±0.22 (↑8%) (23)**	10.3±0.19 (23)	10.2±0.21 (23)	9.8±0.15 (23)	**10.5*±0.20 (↑7%) (23)**	10.0±0.20 (23)	9.8±0.21 (23)
4 [b]	10.3±0.14 (23)	**11.0*±0.22 (↑7%) (23)**	10.2±0.19 (23)	10.3±0.21 (23)	9.8±0.15 (23)	10.5±0.21 (23)	10.0±0.20 (23)	9.8±0.21 (23)
11	26.7±0.42 (23)	27.8±0.39 (23)	25.9±0.40 (23)	26.2±0.56 (23)	25.7±0.40 (23)	26.9±0.40 (23)	25.2±0.39 (23)	25.3±0.53 (23)
17	40.4±0.63 (23)	42.2±0.61 (23)	39.5±0.55 (23)	40.2±0.65 (23)	39.0±0.64 (23)	40.7±0.67 (23)	38.5±0.49 (23)	38.7±0.60 (23)
21	51.1±0.81 (23)	**53.9*±0.73 (↑5%) (23)**	50.3±0.62 (23)	50.4±1.03 (23)	49.4±0.76 (23)	51.9±0.75 (23)	49.0±0.60 (23)	48.6±0.91 (23)
0-21 4[b]-21	45.1 40.8	**47.5** **42,9**	44.2 40.1	44.5 40.1	43.4 38.9	46.2 41.4	43.3 39	43 38.8

Values are mean ± standard error
(# litters) group average body weight data provided in the summary table and appendix are based on the mean for each litter, not for each individual pup..
[a] Before standardization (culling).
[b] After standardization (culling).
*Statistically different from control, Dunnett's test $p \leq 0.05$.
Data obtained from pages 101-103 in the study report.
Last row: weight gain for intervals as calculated by HED reviewer based on data in table.

Post-weaning body weight

There were no statistically significant differences in body weight in offspring post-weaning males and females at any dose level (Table 9).

TABLE 9. Mean (±SD) Post-weaning Pup Body Weights (g)								
Postnatal Day	Dose (ppm							
	0	30	60	180	0	30	60	180
	Males				Females			
28	81.3±5.4 (23)	84.5±6.1 (23)	82.1±5.5 (23)	80.9±6.8 (23)	79.9±5.1 (23)	82.9±5.7 (23)	81.1±5.5 (23)	78.2±5.6 (23)
35	130.9±8.1 (23)	136.3±9.4 (23)	132.0±7.5 (23)	130.0±8.8 (23)	119.1±7.0 (23)	122.8±7.7 (23)	120.0±7.5 (23)	117.4±6.5 (23)
42	180.5±10.0 (23)	186.7±12.4 (23)	181.7±10.1 (23)	179.8±10.4 (23)	146.4±9.0 (23)	150.8±9.3 (23)	147.5±9.4 (23)	145.0±7.2 (23)
49	226.7±13.3 (23)	233.7±15.8 (23)	225.3±12.6 (23)	222.5±13.0 (23)	164.6±10.2 (23)	168.4±10.2 (23)	164.9±11.2 (23)	161.5±7.5 (23)
56	275.2±16.3 (23)	283.1±19.8 (23)	275.5±12.8 (23)	271.9±15.8 (23)	182.5±11.8 (23)	186.9±11.5 (23)	185.3±13.1 (23)	182.1±8.9 (23)
63	312.7±19.5 (23)	320.7±22.9 (23)	312.5±13.9 (23)	308.7±18.2 (23)	196.3±12.8 (23)	200.3±11.8 (23)	196.1±13.9 (23)	193.9±9.5 (23)
70	342.9±22.4 (23)	351.2±25.4 (23)	344.0±15.2 (23)	336.7±20.6 (23)	207.9±13.9 (23)	214.4±12.7 (23)	210.1±14.8 (23)	205.5±9.5 (23)

Values are mean ± standard error
(# litters) group average body weight data provided in the summary table and appendix are based on the mean for each litter, not for each individual pup..
Actual days of measurements occurred within the week of PND 28,35,42,49,56,63,70
Values were not statistically different from control, p≤0.05.
Data were obtained from Table 15 pages 112-113 of the study report

3. Developmental Landmark

Sexual maturation and surface righting results are presented in Table 10. No treatment-related effects were observed on sexual maturation. Times to preputial separation and vaginal opening were similar between the treated groups and controls. There was a slight delay in balanopreputial separation in high-dose males (44.9 versus 43.3 for controls). This slight difference from control was not considered to be test substance-related but more likely due to normal variability since the delay was not statistically significant, was within the range (42.2-44.9 days) of historical control for the last 10 studies conducted in Xenometrics laboratory (Sheets et. al., 2005-2008) and was not seen in the two-generation reproduction study that was performed in this laboratory at the same dietary levels (Young, Study no. 08-R72-OB).

Pupil constriction in response to a penlight was apparent in all control and treated pups on PND 21. Therefore, there was no indication of a test substance-related effect at any dietary level.

TABLE 10. Mean (±SE) Age of Sexual Maturation and Surface Righting (days)				
Parameter	Dose (ppm)			
	0	30	60	180
Number of litters (M/F)	23/23	23/23	23/23	23/23
Balanopreputial separation % Pups Reaching Criteria	43.3±0.47 (100)	43.3±0.61 (100)	43.5±0.48 (100)	44.9±0.58 (100)
Vaginal opening % Pups Reaching Criteria	31.5±0.39 (100)	32.0±0.29 (100)	32.0±0.44 (100)	32.3±0.50 (99)
Surface Righting % Pups Reaching Criteria	5.8±0.18 (100)	5.5±0.18 (100)	5.9±0.18 (100)	5.7±0.19 (100)
Pupil Constriction % Pups Reaching Criteria	21.0±0.0 (100)	21.0±0.0 (100)	21.0±0.0 (100)	21.0±0.0 (100)

Values are mean ± standard error
Values were not statistically different from control p≤0.05
Data were obtained from Table 14 on page 110 of the study report

4. Behavioral Assessment

a. Functional observational battery

In males, no treatment-related effects were observed during the functional observational battery at any dose level. Incidental findings in males observed on various test days were brown perianal stain (one low-dose male on PND 35), red lacrimal stain (one low-dose on PND 45), exophthalmia, (one mid-dose on PND 45 and 60, with pupil not visible on PND 60), dermal lesion described as a scab (one low-dose on PND 35 and 45, one control and one low-dose each on PND 60 and three mid-dose on PND 60) and alopecia (one mid-dose on PND 60) (Table 11a).

TABLE 11a. Functional Observational Results (Incidence) in Males

Observations	Dose (ppm)			
	0	30	60	180
PND 4				
No Findings	20(100) 0(0)	20(100) 0(0)	20(100) 0(0)	20(100) 0(0)
PND 11				
No Findings	20(100) 0(0)	20(100) 0(0)	20(100) 0(0)	20(100) 0(0)
PND 21				
No Findings	20(100) 0(0)	20(100) 0(0)	20(100) 0(0)	20(100) 0(0)
PND 35				
Observation During Handling (Severity) Not Observed (0) Brown Perianal Stain (2)	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)	 20(100) 0(0)
Observation During handling Not Observed Scab	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)	 20(100) 0(0)
PND 45				
Observation During Handling (Severity) Not Observed (0) Red Lacrimal Stain (1)	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)	 20(100) 0(0)
Observation During Handling Not Observed (0) Exophthalmia	 20(100) 0(0)	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)
Observation During Handling Not Observed Scab	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)	 20(100) 0(0)
PND 60				
Handling-Other, Alopecia Not Observed Present	 20(100) 0(0)	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)
Observation During Handling Not Observed Exophthalmia	 20(100) 0(0)	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)
Observation During Handling Not Observed Scab	 19(95) 1(5)	 19(95) 1(5)	 17(85) 3(15)	 20(100) 0(0)
Pupil Size Normal Not Performed	 20(100) 0(0)	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)
Pupil Response Dilated then constrict with penlight Left pupil not visible	 20(100) 0(0)	 20(100) 0(0)	 19(95) 1(5)	 20(100) 0(0)

Values represent the number of animals and % incidence (in parentheses) with or without observation
Values were not statistically different from control, $p \leq 0.05$
Data were obtained from Table 17 on pages 140-167 in the study report

In females, there were no treatment related FOB findings at any dose level. The mean number of rears in the open field was significantly ($p \leq 0.05$) increased by 60% in the 30 ppm female group on PND 45 (Table 11b). This difference from control is not considered to be test substance-related since the incidence was not dose related and the mean average for rears in the control animals (4.3) is below the range for historical controls data (Table 12). In addition, significant increased incidence *of vocalization in the ease of removal* from the home cage was observed in the 180 ppm female group on PND 4, (Table 11b). This difference from control is not considered to be test substance-related by HED reviewers nor the study authors. Additional incidental findings in females on various test days included exophthalmia with dilated pupils that did not respond to penlight (one low-dose on PND 45 and 60), exophthalmia (one low-dose on PND 35, 45 and 60) and a dermal lesion described as a scab (one mid-dose on PND 60).

TABLE 11b: Functional Observational Battery Results (Incidence) in Females

Observations	Dose (ppm)			
	0	30	60	180
PND 4				
Observation During Handling				
Minimal Resistance	20(100)	18(90)	19(195)	**15(75)***
Minimal Resistance with Vocalization	0(0)	2(10)	1(5)	**5(25)**
PND 11				
No Findings	20(100)	20(100)	20(100)	20(100)
	0(0)	0(0)	0(0)	0(0)
PND 21				
No Findings	20(100)	20(100)	20(100)	20(100)
	0(0)	0(0)	0(0)	0(0)
PND 35				
Observation During Handling				
Not Observed	20(100)	19(95)	20(100)	20(100)
Exophthalmia	0(0)	1(5)	0(0)	0(0)
PND 45				
Observation During Handling				
Not Observed	20(100)	18(90)	20(100)	20(100)
Exophthalmia	0(0)	2(10)	0(0)	0(0)
Rearing, Mean ± S.D.	4.3±2.1	**6.9±2.4*** **(↑60%)**	5.4±3.1	6.2±2.7
Pupil Size				
Normal	20(100)	19(95)	20(100)	20(100)
Dilated	0(0)	1(5)	0(0)	0(0)
Pupil Response				
Dilated then constrict with penlight	20(100)	19(95)	20(100)	20(100)
Dilated with no response to penlight	0(0)	1(5)	0(0)	0(0)
PND 60				
Observation During Handling				
Not Observed	20(100)	18(90)	20(100)	20(100)
Exophthalmia	0(0)	2(10)	0(0)	0(0)
Observation During handling				
Not Observed	20(100)	20(100)	19(95)	20(100)
Scab	0(0)	0(0)	0(5)	0(0)
Pupil Size				
Normal	20(100)	19(95)	20(100)	20(100)
Dilated	0(0)	0(5)	0(0)	0(0)
Pupil Response				
Dilated then constrict with penlight	20(100)	19(95)	20(100)	20(100)
Dilated with no response to penlight	0(0)	0(5)	0(0)	0(0)

Values represent the number of animals and % incidence (in parentheses) with or without observation
* Values statistically different from control, $p \leq 0.05$
Severity; 0= Not Observed, 1= Slight, 2= Moderate to Severe N=20/sex/dose
Data were obtained from Table 17 on pages 168-195 of the study report.

TABLE 12. Historical Control for Rearing in the Open Field	
PND 45 (± 1 day)	
Study Number	Rear (mean±SD)
04-D72-UM	4.9±2.3
04-D72-UE	6.4±2.9
04-D72-VK	8.9±3.4
04-D72-WO	5.2±1.7
04-D72-YE	6.6±2.8
05-D72-YF	5.4±2.1
06-D72-DH	7.1±3.3
06-D72-EV	7.9±2.8
07-D72-IL	9.0±3.8
07-D72-KC	6.4±2.6

b. Motor and locomoter activity

Total motor and locomotor activities are summarized in Tables 13 and 14. There were no treatment-related effects on motor or locomotor activity in either sex at any dose level. Age-related changes in the levels of motor and locomotor activity were evident in control males and females. On PND 13, the overall 60-minute test sessions for locomotor activity results for the youngest controls were very low compared to subsequent test occasions. This outcome is consistent with the relatively undeveloped ambulatory skills and sensory functions (e.g., eyelids closed) of the pups. This was followed by a progressive increase in levels of motor and locomotor activity with age. *Gender related differences in activity were apparent on PND 60 only, with modestly higher levels of motor and locomotor activity for females, compared with males.* These comparisons (within the control group) describing performance by age and gender were not subjected to statistical analysis.

TABLE 13. Mean (±S.D) Motor Activity Data (total activity counts for session)				
Test Day	Dose (ppm)			
	0	30	60	180
Males				
PND 13	70±70 (20)	57±46 (20)	57±46 (20)	84±79 (20)
PND 17	182±81 (20)	219±129 (20)	209±103 (20)	182±95 (20)
PND 21	281±102 (20)	300±117 (20)	330±147 (20)	302±151 (20)
PND 60	485±103 (20)	474±99 (20)	545±109 (20)	461±96 (20)
Females				
PND 13	88±64 (20)	73±46 (20)	101±90 (20)	108±85 (20)
PND 17	192±120 (20)	206±109 (20)	213±115 (20)	203±160 (20)
PND 21	290±161 (20)	312±120 (20)	355±106 (20)	339±214 (20)
PND 60	691±168 (20)	630±114 (20)	695±145 (20)	744±271 (20)

Values are mean ± standard deviation (n)
Values were not statistically different from control, $p \leq 0.05$
Data were obtained from Table 18 on pages 197-198 of the study report

TABLE 14. Mean (±S.D) Locomotor Activity Data (total activity counts per session)				
Test Day	Dose (ppm)			
	0	30	60	180
Males				
PND 13	6±13 (20)	8±13 (20)	5±4 (20)	8±11 (20)
PND 17	43±23 (20)	56±36 (20)	50±29 (20)	41±62 (20)
PND 21	85±26 (20)	89±41 (20)	87±31 (20)	79±26 (20)
PND 60	332±88 (20)	335±85 (20)	390±94 (20)	320±76 (20)
Females				
PND 13	10±12 (20)	8±9 (20)	13±30 (20)	12±16 (20)
PND 17	48±34 (20)	54±33 (20)	54±30 (20)	52±39 (20)
PND 21	85±43 (20)	88±30 (20)	100±29 (20)	104±66 (20)
PND 60	460±138 (20)	418±100 (20)	439±95 (20)	484±188 (20)

Values are mean ± standard deviation (n)
Values were not statistically different from control, $p \leq 0.05$
Data were obtained from Table 19 on pages 200-201of the study report.

Motor and locomotor activity data were also subjected to analysis at each 10-min interval of the 60-min test session. Evaluation of the progressive decrease in activity over the course of a test session provides a measure of habituation. For motor activity, habituation was evident in control males and females at all ages. For locomotor activity, habituation was apparent in controls at all ages except PND 13, when locomotor activity was quite low for all six intervals of the test session.

An analysis of the data by test interval provided no evidence of a test substance-related effect at any dietary level in either sex. The levels of motor and locomotor activity were generally comparable to control for all test intervals on all test occasions.

c. Auditory startle habituation

There were no statistically significant differences in startle amplitude for treated males or females, relative to controls, at any dietary level. The average response amplitude for treated animals for all 50 trials and the response amplitude for the five blocks of trials, which is used to assess habituation, were comparable to control at all dietary levels (Table 15).

The amplitude of the startle response increased with age in both sexes. This reflects a true age-related increase in the force of the response, since body weight is not included in the measure of response amplitude (see Methods). The average response amplitude on PND 23 and 60 (± 2 days) was 34 g and 170 g, respectively, for control males, and 33 g and 113 g, respectively, for control females. These results also reflect a gender difference in response amplitude on PND 60. Habituation was apparent in control males and females as a decrease in response amplitude over the course of the test session. However, these comparisons (within the control group) to describe performance by age were not subjected to statistical analysis.

TABLE 15. Mean (±SD) Auditory Startle Reflex Peak Amplitude Data					
Time	Block	Dose (ppm)			
		0	30	60	180
Males					
PND 23	Block 1	37±15	38±19	42±15	36±18
	Block 2	37±14	33±18	41±15	36±15
	Block 3	35±17	30±16	39±13	32±17
	Block 4	31±15	30±14	39±12	31±12
	Block 5	30±15	28±14	33±13	30±15
	Avg. for Total Session	**34±14**	**32±15**	**39±12**	**33±13**
	No. of Animals	20	19	20	20
	Body Weight	57	61	59	57
PND 60	Block 1	212±99	248±128	271±116	217±140
	Block 2	209±72	259±156	269±157	232±153
	Block 3	185±82	216±124	225±120	219±146
	Block 4	131±66	164±86	184±93	156±116
	Block 5	114±61	124±67	154±85	140±96
	Avg. for Total Session	**170±66**	**202±99**	**221±100**	**193±123**
	No. of Animals	20	18	20	20
	Body Weight	292	304	294	297
Females					
PND 23	Block 1	38±15	36±13	44±18	33±15
	Block 2	36±15	36±17	43±20	36±17
	Block 3	32±14	30±13	36±12	34±16
	Block 4	30±14	27±13	33±13	32±14
	Block 5	28±15	26±11	33±15	27±13
	Avg. for Total Session	**33±13**	**31±12**	**38±13**	**32±14**
	No. of Animals	20	20	20	19
	Body Weight	57	58	57	56
PND 60	Block 1	121±76	103±57	148±72	129±64
	Block 2	140±93	113±78	142±97	140±92
	Block 3	121±71	105±74	122±98	112±61
	Block 4	103±62	85±52	110±74	92±54
	Block 5	81±51	75±53	97±53	84±50
	Avg. for Total Session	**113±62**	**96±56**	**124±71**	**111±52**
	No. of Animals	20	20	20	20
	Body Weight	192	192	191	185

Values are mean ± standard deviation
Values were not statistically different from control, $p \leq 0.05$
Data were obtained from Tables 22 (pages 221) and 23 (pages 224-227) of the study report.

d. Learning and memory testing

1. Postweaning passive avoidance

The acquisition and retention data for passive avoidance performance are summarized in Table 16. No treatment-related differences in learning or memory were observed in any treated group relative to controls in the passive avoidance test. Acquisition and retention were clearly evident in control males and females. On the first test occasion, acquisition was evident in males and females as a marked increase in the latency to cross for the second trial (an average 177.0 and 177.9 seconds, respectively), compared to the first trial (an average 58.2 and 42.2 seconds, respectively). Thus, acquisition of the avoidance response (a failure to cross within the 180-seconds time limit for a trial) was quickly attained in control males and females. On the second test occasion, which occurred one week after the first, retention was evident in control males and females as a protracted delay to cross within the 180-second time limit of the first trial (an average 172.1 and 176.7 seconds, respectively), compared to the first trial on the first test day (an average 58.2 and 42.2 seconds, respectively). Retention was also evident in control males

and females by a reduced average number of trials-to-criterion on the second test occasion (2.1 and 2.3 trials, respectively), compared to the first (3.1 and 3.0 trials, respectively). These comparisons (within the control group) to verify changes in performance with experience (acquisition/learning and retention/memory) were not subjected to statistical analysis.

As per standard procedure, animals that either failed to satisfy the criteria used to establish acquisition or that failed to cross during the first two trials (and therefore never received the conditioning stimulus) were not tested for retention. There was only one control and one low-dose female and no males that did not cross during the learning phase.

TABLE 16. Passive Avoidance Performance at PND 23 and 30 Offspring (mean ± S.D.)

Session	Parameter	Dose (ppm)			
		0	30	60	180
Males					
Session 1 (Learning Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	3.1±0.3	3.2±0.4	3.1±0.3	3.3±1.0
	Latency trial 1 (seconds)	58.2±49.3	43.6±34.3	57.9±48.6	34.4±35.7
	Latency trial 2 (seconds	177.0±12.2	166.1±32.8	177.8±8.8	159.8±55.5
	Failed to Meet Criterion	0(0%)	0(0%)	0(0%)	0(0%)
	Failed to Cross During Learning Phase	0(0%)	0(0%)	0(0%)	0(0%)
Session 2 (Retention Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	2.1±0.3	2.1±0.3	2.3±0.7	2.3±0.4
	Latency trial 1 (seconds)	172.1±31.4	165.2±44.4	178.3±7.0	156.8±54.2
	Latency trial 2 (seconds)	180.0±0.0	180.0±0.0	174.4±22.0	180.0±0.0
Females					
Session 1 (Learning Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	3.0±0.4	2.9±0.3	3.1±0.3	3.0±0.0
	Latency trial 1 (seconds)	42.2±51.1	32.1±42.2	36.0±26.8	30.4±27.4
	Latency trial 2 (seconds	177.9±8.4	180.0±0.0	172.2±31.1	180.0±0.0
	Failed to Meet Criterion	0(0%)	0(0%)	0(0%)	0(0%)
	Failed to Cross During Learning Phase	1(6%)	1(6%)	0(0%)	0(0%)
Session 2 (Retention Phase)	Number of Animals Tested	16	15	16	16
	Number of Animals Included in Analysis	15	15	16	16
	Trials to criterion	2.3±0.6	2.2±0.6	2.3±0.6	2.2±0.5
	Latency trial 1 (seconds)	176.7±8.0	178.2±7.0	172.3±21.2	169.6±41.6
	Latency trial 2 (seconds	171.0±34.9	170.0±38.6	178.2±7.4	178.3±6.8

Trials to Criterion = Mean # Trials per Group ± S.D.
Latency to trial 1 = Mean Session 1 duration (seconds) per Group ± S.D.
Latency to trial 2 = Mean Session 2 duration (seconds) per Group ± S.D.
Failed to Meet Criterion= Number of Animals that received the shock but did not demonstrate acquisition.
Failed to Cross = Number of Animals that never received the shock.
Values were not statistically different from control, $p \leq 0.05$
Data were extracted from Table 24 on pages 229-230 of the study report

2. Adult offspring – water maze

There were no differences in acquisition or retention related to test substance treatment in either sex at any dose level (Table 17) in the water maze. An increased number of trials to criterion (acquisition) were measured in high dose males (9.0 versus 6.8 for controls) and females (7.8 versus 6.1 for controls). However, no indication of an effect on retention was indicated. The

differences in acquisition are likely due to normal variability (Table 17) as indicated by a relatively large range of responses within each group and the lack of statistical significance in the high dose males compared to controls. Moreover, other related parameters did not support any apparent effect. For example, passive avoidance testing on PND 23 did not indicate any evidence of an effect on acquisition or retention in either sex at any dietary level tested. Also, other neurobehavioral findings were not evident in any other tests performed. The number of errors for the first trial of acquisition was increased for mid- and high-dose females (1.1 and 1.3 errors, respectively compared to 0.3 errors for controls). These differences were noted in the first trial of the learning phase only, occurred only in one sex, and the mean number errors in the mid- and high-dose groups were more consistent with the historical control range for errors (Table 18).

TABLE 17. Water Maze Performance on PND 60 (± 2 days) and Seven Days Later in Offspring (mean±SD)

Session	Parameter	Dose (ppm)			
		0	30	60	180
Males					
Session 1 (Learning Phase)	Number of Animals	16	16	16	16
	Trial 1 to Criterion (Mean ± S.D.)	6.8±1.7	6.6±2.0	7.1±2.9	**9.0±3.2**
	Trial 1- Errors (Mean ± S.D.)	0.6±1.0	0.3±0.5	0.4±0.6	0.8±0.8
	Trial 1- Duration (seconds) (Mean ± S.D.)	16.9±17.9	12.6±5.4	14.7±13.3	20.9±17.3
	Trial 2- Errors (Mean ± S.D.)	0.7±0.8	0.6±0.9	0.4±0.6	0.5±0.6
	Trial 2- Duration (seconds) (Mean ± S.D.)	15.3±11.4	17.5±18.6	14.8±12.6	18.8±19.9
	Failed to Meet Criterion	0(0%)	0(0%)	1(6%)	1(6%)
Session 2 (Retention Phase)	Number of Animals	16	16	15	15
	Trial 1 to Criterion (Mean ± S.D.)	7.1±2.5	5.5±0.8	5.9±1.9	6.3±2.0
	Trial 1- Errors (Mean ± S.D.)	0.8±0.8	0.9±1.5	0.7±1.2	0.3±0.5
	Trial 1- Duration (seconds) (Mean ± S.D.)	13.2±7.4	15.6±14.5	13.7±17.0	7.8±6.4
	Trial 2- Errors (Mean ± S.D.)	0.1±0.3	0.1±0.5	0.1±0.5	0.2±0.4
	Trial 2- Duration (seconds) (Mean ± S.D.)	5.4±4.3	5.7±7.9	7.5±7.8	4.9±3.7
Females					
Session 1 (Learning Phase)	Number of Animals	16	16	16	16
	Trial 1 to Criterion (Mean ± S.D.)	6.1±1.9	6.7±1.8	6.8±1.6	**7.8±2.0***
	Trial 1- Errors (Mean ± S.D.)	0.3±0.5	0.7±0.9	**1.1±0.9***	**1.3±1.3***
	Trial 1- Duration (seconds) (Mean ± S.D.)	12.8±8.0	19.6±15.4	22.5±14.1	23.3±18.1
	Trial 2- Errors (Mean ± S.D.)	0.3±0.9	0.4±0.6	0.5±0.7	0.7±0.9
	Trial 2- Duration (seconds) (Mean ± S.D.)	11.5±11.2	13.6±9.3	14.8±13.3	17.5±13.3
	Failed to Meet Criterion	0(0%)	0(0%)	0(0%)	0(0%)
Session 2 (Retention Phase)	Number of Animals	16	16	16	16
	Trial 1 to Criterion (Mean ± S.D.)	6.8±2.9	5.9±1.7	7.3±3.3	5.7±2.0
	Trial 1- Errors (Mean ± S.D.)	0.4±0.6	0.3±0.6	0.6±0.8	0.3±0.4
	Trial 1- Duration (seconds) (Mean ± S.D.)	10.6±8.4	10.2±8.3	12.3±9.7	8.9±5.8
	Trial 2- Errors (Mean ± S.D.)	0.4±0.9	0.1±0.3	0.1±0.5	0.1±0.3
	Trial 2- Duration (seconds) (Mean ± S.D.)	6.8±5.0	4.3±1.4	5.0±2.7	4.2±1.6

Values for rats who failed to learn during session 1 were not included in means for session 2.
* Statistically different from control, $p \leq 0.05$
Data were extracted from Table 25 on pages 232-233 of the study report

Acquisition and retention in the water maze test were evident in both control males and females. On the first test-occasion, acquisition was evident in controls as a progressive decrease in the average time to escape (to reach the exit ramp) over successive trials. For males the average trial duration (time to escape) decreased from the first trial (an average of 16.9 seconds) to the

second trial (an average 15.3 seconds). By comparison for females, the average trial duration (time to escape) decreased from the first trial (an average of 12.8 seconds) to the second trial (an average of 11.5 seconds). Further reductions were evident in the time to escape for subsequent trials for both sexes (e.g., an average 6.2 and 5.8 seconds for males and females, respectively, for the fifth trial). On the second test occasion, the number of trials to criterion did not decrease; relative to performance for acquisition; but essentially remained unchanged in males (7.1 versus 6.8 for acquisition) and in females (6.8 versus 6.1 for acquisition). However, retention was evident as there was a shorter trial duration for the first trial, compared to the first trial of acquisition for males (13.2 versus 16.9 seconds for acquisition) and females (10.6 versus 12.8 seconds for acquisition). A slightly lower number of trials to criterion for acquisition is most likely the reason that performance did not improve for acquisition in both sexes (more notable in females). A comparison of these results with historical control data (Table 18) supports this interpretation. These comparisons within the control group to verify changes in performance with experience were not subjected to statistical analysis.

As per standard procedure, animals that failed to demonstrate acquisition were not tested for retention. There were two males (one mid- and one high-dose) that failed to demonstrate acquisition. Remaining males and females at all dietary levels demonstrated acquisition.

TABLE 18. Historical Control for Water Maze Trials To Criterion in Both Sexes and Errors During Acquisition in Females (Mean ± S.D)

Study Number	Males	Females	
	Trials to Criterion During Acquisition (Mean ± S.D.)	Trials to Criterion During Acquisition (Mean ± S.D.)	Errors During Acquisition (Mean ± S.D.)
04-D72-UE	8.2±3.1	7.9±3.1	0.4±0.6
04-D72-UM	6.8±1.4	7.2±1.9	0.7±0.7
04-D72-VK	5.9±1.2	7.4±3.1	1.1±1.3
04-D72-WO	6.9±2.4	8.1±2.1	1.6±1.5
04-D72-YE	7.8±3.1	7.4±3.0	1.3±1.8
05-D72-YF	7.8±2.7	6.7±2.2	0.8±0.9
06-D72-DH	7.6±3.0	7.9±2.3	0.9±0.7
06-D72-EV	6.8±2.6	8.1±2.1	1.2±0.9
06-D72-GL	6.4±1.4	7.6±2.6	0.8±1.0
07-D72-IL	6.8±2.6	5.9±0.9	0.6±0.6
07-D72-KC	7.4±3.3	7.7±2.4	0.9±0.7
Mean ± SD (11 studies)	7.1±0.7	7.4±0.7	0.9±0.3
This study Control	6.8±1.7	6.1±1.9 (low)	0.3±0.5 (low)
High dose	9.0±3.2	7.8±2.0	1.3±1.3

Data were obtained from Text Table 18 on page 56 of the study report

5. Ophthalmology

There were no test substance-related lesions in males or females at any dose level. The corneal opacity observed in males and females from various dietary levels (including control) was considered to be incidental and unrelated to the test substance, due to lack of dose response (in females), consistency by gender and/or because the incidence was within the range of historical controls. The only ophthalmic finding in this study was as follows: Opacity, corneal (**M: 1, 1, 2, 3;** F: 1, 0, 0, 1).

6. Postmortem Results

Gross Pathology

There were no gross observations considered to be test substance-related at any dietary level in either sex for perfused Day 21 or termination animals or in non-perfused termination animals. One high-dose male (3102 1) was sacrificed on 4/21/09 due to injuries that occurred from teeth

caught on either the feeder or on the cage. This animal had been selected for perfusion but was replaced with another animal.

Gross observations not considered test substance-related included "bone, other – malformation" and/or "skin, hindleg – raised zone" due to an extra digit on the right hind foot in one mid-dose male (2119 04) and a high-dose female (3108 10) in perfused PND 21 pups. One perfused termination adult high-dose female (3121 06) had a brain - pitted zone, which was most likely caused by an injury (see Micropathology Section 5.1.3 of pathology report). Two non-perfused termination adult males (control 0111 02 and low-dose 1110 02) and two non-perfused termination females (mid-dose 2119 06 and 2125 08) had kidneys - dilated pelvis. Lastly, one non-perfused termination female (high-dose 3117 08) had a liver median lobe abnormality. None of these findings were considered to be test substance-related since the incidence was small and generally there was no dose-related response.

b. Brain weight and terminal body weight

Mean brain data are summarized in Table 19. There were no treatment related effects on absolute and relative fixed brain weights for terminal perfused males and females and non-perfused males and females at any dose level. In 21-day male pups, absolute mean brain weight for non-perfused terminal was significantly increased by 5% in the 30 ppm group compared to controls (Table 19). This increase was not considered to be test substance-related since there was no dose relationship and is most likely related to the animal at this dose level being slightly larger.

All other values in the treated groups were similar to controls.

TABLE 19. Mean (±SD) Brain Weight Data

Parameter	Dose (ppm)			
	0	30	60	180
Males				
PND 21 (Perfused)				
Terminal Body Weight (g)	50.9±5.0 (10)	54.2±4.0 (10)	49.3±4.1 (10)	50.1±7.5 (10)
Brain, Fixed (g)	1.413±0.053 (10)	1.423±0.069 (10)	1.397±0.091 (10)	1.398±0.078 (10)
Brain, Fixed/Body Weight (%)	2.803±0.293 (10)	2.641±0.234 (10)	2.849±0.282 (10)	2.834±0.370 (10)
PND 75 (±5) (Termination-Perfused)				
Terminal Body Weight (g)	359.3±37.8 (10)	375.0±20.1 (10)	356.2±22.2 (10)	336.0±30.5 (10)
Brain, Fixed (g)	1.863±0.087 (10)	1.909±0.055 (10)	1.877±0.093 (10)	1.860±0.045 (10)
Brain, Fixed/Body Weight (%)	0.523±0.050 (10)	0.510±0.023 (10)	0.529±0.046 (10)	0.557±0.043 (10)
PND 75 (±5) (Termination-Non-Perfused)				
Terminal Body Weight (g)	344.3±24.8 (10)	363.0±28.9 (10)	342.0±25.4 (10)	339.0±25.5 (10)
Brain, Fixed (g)	1.937±0.071 (10)	**2.025±0.093* (↑5%) (10)**	1.921±0.074 (10)	1.944±0.075 (10)
Brain, Fixed/Body Weight (%)	0.565±0.042 (10)	0.561±0.057 (10)	0.564±0.048 (10)	0.575±0.030 (10)
Females				
PND 21 (Perfused)				
Terminal Body Weight (g)	50.2±4.6 (10)	51.6±2.5 (10)	50.1±2.7 (10)	49.3±4.1 (10)
Brain, Fixed (g)	1.348±0.034 (10)	1.391±0.087 (10)	1.352±0.043 (10)	1.370±0.034 (10)

TABLE 19. Mean (±SD) Brain Weight Data				
Parameter	Dose (ppm)			
	0	30	60	180
Brain, Fixed/Body Weight (%)	2.710±0.287 (10)	2.703±0.219 (10)	2.707±0.148 (10)	2.793±0.0246 (10)
PND 75 (±5) (Termination-Perfused)				
Terminal Body Weight (g)	213.0±17.3 (10)	214.4±17.3 (10)	210.6±19.9 (10)	207.0±11.9 (10)
Brain, Fixed (g)	1.724±0.057 (10)	1.799±0.068 (10)	1.743±0.049 (10)	1.747±0.085 (10)
Brain, Fixed/Body Weight (%)	0.815±0.078 (10)	0.842±0.050 (10)	0.835±0.086 (10)	0.845±0.047 (10)
PND 75 (±5) (Termination-Non-Perfused)				
Terminal Body Weight (g)	208.8±14.6 (10)	214.2±15.0 (10)	218.0±17.8 (10)	208.5±15.8 (10)
Brain, Fixed (g)	1.805±0.068 (10)	1.849±0.098 (10)	1.871±0.078 (10)	1.806±0.064 (10)
Brain, Fixed/Body Weight (%)	0.866±0.036 (10)	0.865±0.039 (10)	0.861±0.051 (10)	0.869±0.059 (10)

Values are mean ± standard deviation (n)
*Statistically different from control, p≤0.05
Data were obtained from Table 4A OW1K-SUM, Table 5A OW2K-SUM and Table 6A OW3K-SUM in the pathology report.

c. Brain measurement morphometry

Gross measurements. As indicated in Table 20, there were no test substance- related differences from control in mean cerebrum or mean cerebellum lengths for *gross* brain measurements for either PND Day 21 or PND 75 (±5) for either sex.

TABLE 20. Histopathology Findings (Initial)				
Parameter	Dose (ppm)			
	0	30	60	180
Males				
Gross Measurements				
Day 21				
Ant/Post Cerebrum Length (mm)	13.67±0.27(10)	13.86±0.28(10)	13.70±0.32(10)	13.68±0.14(10)
Ant/Post Cerebrum (mm)	7.39±0.48(10)	7.31±0.48(10)	7.31±0.17(10)	7.32±0.30(10)
PND 75 (±5) (Termination-Perfused)				
Ant/Post Cerebrum Length (mm)	14.69±0.23(10)	14.90±0.26(10)	14.80±0.33(10)	14.55±0.39(10)
Ant/Post Cerebrum (mm)	8.19±0.27(10)	8.35±0.30(10)	8.14±0.56(10)	8.10±0.34(10)
Females				
Gross Measurements				
PND 21				
Ant/Post Cerebrum Length (mm)	13.50±0.23(10)	13.63±0.21(10)	13.63±0.24(10)	13.69±0.15(10)
Ant/Post Cerebrum (mm)	7.15±0.28(10)	7.27±0.43(10)	7.25±0.26(10)	7.09±0.28(10)
PND 75 (±5) (Termination-Perfused)				
Ant/Post Cerebrum Length (mm)	14.07±0.34(10)	14.42±0.35(10)	14.36±0.42(10)	14.42±0.32(10)
Ant/Post Cerebrum (mm)	8.15±0.40(10)	8.28±0.28(10)	8.02±0.31(10)	8.25±0.30(10)

Micropathology brain measurements. As indicated in table 21, there were no indications of treatment related differences in *males* with regard to micropathology brain measurements when the high dose is compared to the controls. However, Table 21 indicates that the high dose females demonstrated statistical differences in the frontal and parietal cortexes and in the caudate putamen reflecting possible 10 to 12% increases for the PND 21 but not at the terminal sacrifices.

TABLE 21. Microscopic Histopathology Findings (Initial)				
Parameter	Dose (ppm)			
	0	30	60	180
Males				
PND 21				
Frontal Cortex (mm)	1.832±0.010(10)	-	-	1.804±0.005(9)
Parietal Cortex (mm)	1.880±0.011(10)	-	-	1.905±0.006(9)
Caudate Putamen (mm)	2.704±0.059(10)	-	-	2.700±0.108(9)
Hippocampal Gyrus (mm)	1.701±0.007(10)	-	-	1.719±0.012(10)
Cerebellum (mm)	5.007±0.020(10)	-	-	4.970±0.230(10)
PND 75 (±5) (Termination-Perfused)				
Frontal Cortex (mm)	1.799±0.014(9)	-	-	1.803±0.015(9)
Parietal Cortex (mm)	1.985±0.015(9)	-	-	2.000±0.014(9)
Caudate Putamen (mm)	3.266±0.029(9)	-	-	3.216±0.039(9)
Hippocampal Gyrus (mm)	1.948±0.014(9)	-	-	1.975±0.019(10)
Cerebellum (mm)	5.575±0.144(10)	-	-	5.576±0.088(10)
Females				
PND 21				
Frontal Cortex (mm)	1.636±0.003(10)	-	-	**1.807±0.007*(9) (↑10%)**
Parietal Cortex (mm)	1.702±0.003(10)	-	-	**1.906±0.005*(9) (↑12%)**
Caudate Putamen (mm)	2.539±0.039(10)	-	-	**2.805±0.031*(9) (↑10%)**
Hippocampal Gyrus (mm)	1.580±0.008(10)	-	-	1.621±0.016 (10)
Cerebellum (mm)	4.995±0.118(10)	-	-	4.900±0.142 (10)
PND 75 (±5) (Termination-Perfused)				
Frontal Cortex (mm)	1.821±0.002(10)	-	-	1.860±0.011 (9)
Parietal Cortex (mm)	1.955±0.003(10)	-	-	1.948±0.014 10)
Caudate Putamen (mm)	3.227±0.022(10)	-	-	3.312±0.033 (10)
Hippocampal Gyrus (mm)	1.821±0.021(10)	-	-	1.778±0.014 (9)
Cerebellum (mm)	5.073±0.068(10)	-	-	4.958±0.176 (10)

Values are mean ± standard deviation (n)
- = not evaluated
* Statistically different from control, p≤0.05
Data obtained from table 4A.OW1K-SUM, Table 5A. OW2K-SUM, Table 7A. BM1-SUM and Table 1QA. BM2-SUM in the pathology report

Recutting. In order to resolve the issue of an apparent effect of propineb on the morphometrics, recutting of the tissues for the PND 21 females was done. Table 22 shows the results of this recutting and compares the original cutting with the recutting. There were statistical differences reflecting apparent increases of up to 17% for the low and mid dose groups but the high dose group had either the smallest apparent increase or was not statistically significantly increased compared to the control. Thus there is no dose response.

Overall, the Investigator concluded that the statistical and/or nonstatistical differences in measurements from treated Day 21 females were considered to be likely due to random biologic variation coupled with greater or lesser degrees of tissue section shrinkage.

HED reviewers concur that there since there is no dose response and because the control group appears low relative to the historical control and because the treated groups are within the historical control range, there is no convincing case that the statistical differences are related to treatment.

It is noted that the recuts are lower in value than the original readings for all comparisons. The difference for the caudate putamen reached 20-22% lower for the recut samples.

TABLE 22. Comparions of the Original and Recutting Histopathology Findings for Females					
Parameter	Dose (ppm)				Historical
	0	30	60	180	Control[a]
Microscopic Measurements – PND 21 (mm)					
Frontal Cortex (mm) Original	1.636±0.003(10)	-	-	**1.807±0.007*(9) (↑10%)**	
Frontal Cortex (mm) Recut	1.476±0.004(10) [↓9.8%][b]	**1.617±0.006*(10) (↑10%)**	**1.568±0.006*(10) (↑6%)**	1.542±0.005(8) (↑4.5%) [↓15%]	1.64-1.98
Parietal Cortex Original	1.702±0.003(10)	-	-	**1.906±0.005*(9) (↑12%)**	
Parietal Cortex Recut	1.570±0.006(10) [↓7.7%]	**1.769±0.006*(10) (↑13%)**	**1.727±0.009*(10) (↑10%)**	**1.706±0.004*(8) (↑9%) [↓10.5%]**	1.70-2.06
Caudate Putamen Original	2.539±0.039(10)	-	-	**2.805±0.031*(9) (↑10%)**	
Caudate Putamen Recut	2.032±0.021(10) [↓20%]	**2.371±0.29*(8) (↑17%)**	**2.371±0.035*(10) (↑17%)**	**2.186±0.005*(8) (↑8%) [↓22%]**	2.83-3.27

[a] From page 21 of 323 (Pathology report) or page 1038.
[b] Number in [ ] is the comparision of the origanl cut with the recut

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS

Test substance-related effects attributed to exposure to Propineb were as follows:

Maternal

30 ppm (2.3 mg/kg bw/day) - There were no test substance-related findings during gestation or lactation.
60 ppm (4.4 mg/kg bw/day) - There were no test substance-related findings during gestation or lactation.
180 ppm (12.3 mg/kg bw/day) – There were no test substance-related findings during gestation or lactation.

The maternal no-observed-adverse-effect level (NOAEL) in the present study is 180 ppm. (equivalent to 12.3 mg/kg bw/day)

Offspring
30 ppm (2.3 mg/kg bw/day) - There were no test substance-related findings.
60 ppm (4.4 mg/kg bw/day) - There were no test substance-related findings.
180 ppm (12.3 mg/kg bw/day) - There were no test substance-related findings.

The offspring NOAEL is 180 ppm (equivalent to 12.3 mg/kg bw/day in the parents)

B. REVIEWER COMMENTS

Maternal (P-generation)
In maternal animals, no test substance-related effects were observed on mortality, clinical signs of toxicity or functional observational battery during gestation or lactation at any dose level. Clinical sign findings that are considered incidental and unrelated to the test substance were

apparent in the 180 ppm females during lactation as red vaginal discharge, lacrimal stain, nasal stain and dehydration and areas of hair loss. These findings were not considered to be treatment-related because incidence was generally low and findings were seen in the control as well as treatment groups. Body weight and food consumption during gestation and lactation were not different from controls at any dose level. Propineb did not induce adverse effects on reproductive performance of animals at any dose level.

The maternal NOAEL is 180 ppm (equivalent to 12.3 mg/kg/day). A maternal LOAEL was not established by this study.

Offspring (F_1 generation)
In offspring, no treatment-related effects were observed on litter size, viability, clinical signs, developmental landmarks, functional observational battery, auditory startle reflex, learning and memory testing, ophthalmology, nervous system morphometric evaluation, or gross or microscopic pathology.

An apparent increase ($P \leq 0.05$) in offspring body weights was noted in the *low-dose* (30 ppm) males and females up to PND 4 (7-8% in males pre- and post culling and 7% in females pre-culling). In addition, body weight was statistically increased in the 30 ppm males on PND 21 (5%) and body weight gain for PND 11-21 was statistically increased in low-dose males (7%) and females 6%). These differences in body weight and body weight gain are not considered treatment-related since there was no relationship to dose and were only seen in the low-dose (30 ppm) animals. Also, in postweaning male and female rats, body weights were similar to controls.

There was a slight delay in balanopreputial separation in *high-dose* males (44.9 versus 43.3 for controls). This slight difference from control was not considered to be test substance-related but more likely due to normal variability, since the delay in this developmental landmark was not statistically significant and was within the range (42.2-44.9 days) of historical control data.

There was a statistical increase in the mean number of rearings in the open field compared to controls for *low-dose* (30 ppm) females on PND 45 (6.9 vs. 4.3 for controls). This difference from control is not considered test substance-related since the incidence was not dose-related and the mean average for rearings in the control animals (4.3) is below the range (4.9-9.0) of historical control data. There was a statistical difference in the ease of removal from the home cage (increased incidence of vocalization) for high-dose (180 ppm) female pups on PND 4 relative to controls. This difference from control is not considered test substance-related since it was not dose-related, the incidence was low and only occurred in one sex.

In water maze performance on PND 60 (±2 days), the number of errors for the first trial of acquisition was increased ($P \leq 0.05$) for *mid- and high*-dose females (1.1 and 1.3 errors, respectively compared to 0.3 errors for controls). These differences were noted in the first trial of the learning phase only, occurred only in one sex, and the mean number of errors in the mid- and high-dose groups were more consistent with the historical control range for errors (0.4-1.6). Thus, the control group was considered lower than expected.

In PND 75 male pups, the non-perfused terminal absolute mean brain weight was significantly increased by 5% in the 30 ppm group. This increase was not considered test substance-related because there was no dose relationship in the terminal nonperfused male brain weights when compared to the other dose levels.

The initial cuttings for Day 21 female pup micropathology brain measurements from the frontal cortex, parietal cortex, and caudate putamen were statistically significantly increased at the 180

ppm dose level over controls. -These differences were not considered treatment related because recutting of these sections indicated that the low and mid dose groups were also higher and the highest differences were in the lower dose group. Thus there was no dose response. Comparison of the data with the historical control data base indicated that the control readings were low relative to the treated groups to further support the contention that the differences were random variation and artifacts of sample preparation.

The offspring NOAEL is 180 ppm (equivalent to 12.3 mg/kg/day). An offspring LOAEL was not established by this study.

Tabular Summary:

Dosage (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Test Substance-Related Effects at LOAEL
P-generation females: 0, 2.3, 4.4 and 12.3	12.3	Not established [>12.3]	Not applicable
F_1-generation: 0, 2.3, 4.4 and 12.3	12.3	Not established [>12.3]	Not applicable

This study is classified as **acceptable/non-guideline** and satisfies the guideline requirements (OPPTS 870.6300; PMRA DACO 4.5.14; OECD 426) for a developmental neurotoxicity study in rats.

This study is classified as **acceptable/non-guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, 83-6); OECD 426 at this time pending a comprehensive review of all available positive control data. Although the study did not demonstrate any effects of treatment, the study is still classified as acceptable since repetition of the study is not believed to contribute additional information.

C. PROTOCOL DEVIATIONS

The minor deviations reported as footnotes in the study report (pages 20, 25, 26, 28 and 29) did not significantly alter the outcome of the study.

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